

Effect of hot water and lactic acid decontamination on *Escherichia coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork

Pia Christiansen^{*(1)}, Rikke Krag⁽²⁾, Søren Aabo⁽¹⁾

⁽¹⁾National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg

⁽²⁾Faculty of Life Sciences, University of Copenhagen, Grønnegårdsvej 15, DK-1870 Frederiksberg C

*Corresponding author: pichr@food.dtu.dk

Abstract

Future progress in food safety of pork meat in Denmark may depend on implementation of carcass decontamination at the slaughter line. The objective of this study was to evaluate the microbial reducing effect of hot water and lactic acid for post harvest carcass decontamination of pork. Application of hot water (80°C) and 1% and 2.5% lactic acid (55°C and 80°C) decontamination after treatment for 0, 5 and 15 s was investigated in laboratory scale using a watering device. The microbial reducing effect was quantified on a mixed flora of stationary phase cultures of *E. coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica* inoculated (10^7 cfu per cm^2 of each of the species) on the skin and meat surface of pork jowl samples (10x10 cm). Mean reductions of 1.0-5.8 \log_{10} units per cm^2 were obtained depending on the different decontamination parameters applied. Increased microbial reductions were observed at higher treatment temperature, longer treatment time, and in the presence of and at higher concentration of lactic acid. In general, higher reductions were observed on the skin surface compared to the meat surface. The observed difference between surfaces seemed to some extent equalised by application of lactic acid. *E. coli* tended to be more sensitive than *S. Typhimurium* and *Y. enterocolitica* to decontamination. Decontamination with 1% lactic acid at 80°C for 5 s showed reductions comparable to hot water decontamination at 80°C for 15 s, indicating a potential cut in water usage. Hot water decontamination at 80°C for 15 s showed mean reductions of 3.1-3.6 \log_{10} units per cm^2 on the skin surface and 2.0-2.8 \log_{10} units per cm^2 on the meat surface. Application of 1% lactic acid at 80°C for 15 s showed mean reductions of 4.5-5.0 \log_{10} units per cm^2 on the skin surface and 3.2-4.4 \log_{10} units per cm^2 on the meat surface, thus showing an additive effect of 1-1.5 \log_{10} units per cm^2 obtained by adding 1% lactic acid to 80°C water.

Introduction

A national *Salmonella* surveillance and control programme in pigs was introduced in Denmark in 1995. However, Danish fresh meat surveillance data on pork indicate that the prevalence of *Salmonella* contaminated carcasses at the slaughter has now reached a plateau around 1.2 %, despite the continued effort to control salmonella. Future progress in food safety of pork meat in Denmark may therefore depend on implementation of post harvest carcass decontamination of pork at the slaughter line (Hurd *et al.* 2008). E.C. regulation 853, article 3, 2004, gives possibility for physical and chemical decontamination in EU. General decontamination of fresh meat has been allowed if potable water is used, while chemical methods for decontamination (e.g. lactic acid) require approval by the EU and EFSA (Hugas and Tsigarida, 2008). In Denmark, commercial hot water decontamination with 80°C for 15 s has been applied for the last decade to pig carcasses with a particular salmonella risk.

Microbial contamination of pig carcasses during slaughtering is an undesirable but unavoidable part of meat processing (Dickson and Anderson, 1992). Different decontamination technologies (physical, chemical and biological) have been investigated and reviewed for the past decades (Dickson and Anderson, 1992; Smulders and Greer, 1998; Sofos and Smith, 1998; Hugas and Tsigarida, 2008). Typically, bacterial reductions of 1-3 \log_{10} have been achieved in model as well as in commercial investigations. Although, some decontamination studies have been performed on pork (Biemuller *et al.*, 1973; Gill *et al.*, 1995 and 1997; Jensen and Christensen, 2000; Eggenberger-Solarzano *et al.*, 2002; Sommers *et al.*, 2002), far the most decontamination studies have been performed on beef. Application of multiple sequential interventions for decontamination of meat has been studied and additive effects have been reported (Eggenberger-Solarzano *et al.*, 2002; Sommers *et al.*, 2002).

The objective of the present study was to evaluate the microbial reducing effect of hot water (80°C) and 1% and 2.5% lactic acid (55°C and 80°C) decontamination for 0, 5 and 15 s on *E. coli*, *Salmonella* Typhimurium and *Yersinia enterocolitica* inoculated on skin and meat surface of pork jowl. Instead of applying the hot water and lactic acid in two different process steps, the additive effect of increasing the temperature of the lactic acid solution to 80°C was investigated in this study.

Materials and Methods

Bacteria strains and inoculum preparation. *Escherichia coli* DH5- α (MS21696), *Salmonella* Typhimurium 4/74 (MS21697) and *Yersinia enterocolitica* 123 (MS21698) from our laboratory collection were propagated from frozen (−80°C) stocks by streaking on BHI agar and incubating aerobically at 37°C for 24 h. From BHI agar, colonies were inoculated in 10 mL BHI broth and incubated aerobically at 37°C for 18–22 h until stationary phase was reached. For preparation of bacteria inoculum, the three bacteria cultures of *E. coli*, *S. Typhimurium* and *Y. enterocolitica* were mixed in the ratio 1:1:1, obtaining a mixed culture inoculum with 10⁹ CFU/mL of each bacterial species.

Meat samples preparation. Fresh pork jowls, taken from carcasses at the end of the slaughter line, immediately before chilling, were collected at the day of trial from a large slaughter plant in DK. The pork jowls were trimmed and cut into sample pieces of 10x10 cm. Pork samples were artificial surface inoculated with 100 μ l mixed inoculum and the inoculum was evenly spread on the surface with a sterile spatula (inoculation level 10⁷ CFU per cm² of each bacteria species). The pork samples were allowed to stand for 30 minutes at room temperature before decontamination treatment to allow attachment.

Decontamination procedure. Sterile water and lactic acid solutions of 1% and 2.5% (v/v) lactic acid were prepared. The temperature of the decontamination solutions was adjusted to 55°C \pm 1°C or 80°C \pm 1°C prior to application. Pork samples were placed vertically and poured with the decontamination solution by a watering device in order to imitate in-line cabinet hot water carcass decontamination with water sheets.

Microbiological analysis. After decontamination, the samples were aseptically transferred to a sampling bag containing 100 ml 0.9% NaCl solution and stomached for 2 min at high speed. The pork sample rinses were serially diluted and appropriate dilutions were plated on Petrifilm, Xylose Lysine Deoxycholate (XLD) agar, and Cefsulodin Irgasan Novobiocin (CIN) agar for the enumeration of *E. coli*, *S. Typhimurium* and *Y. enterocolitica*, respectively. Plates were incubated aerobically at 37°C for 24 h and characteristic colonies were counted. All microbiological data were transformed into log₁₀ CFU/cm² before calculating mean reductions. Reported means are the average of three independent replications.

Results

Mean reductions of 1.0–5.8 log₁₀ units per cm² of *E. coli*, *S. Typhimurium* and *Y. enterocolitica* were obtained by hot water and lactic acid decontamination (Figure 1). Hot water decontamination at 80°C for 15 s showed mean reductions of 3.6 and 3.2 and 3.1 log₁₀ units per cm² on the skin surface and 2.8 and 2.2 and 2.0 log₁₀ units per cm² on the meat surface for *E. coli*, *S. Typhimurium* and *Y. enterocolitica*, respectively (Figure 1). Decontamination with 1 and 2.5% lactic acid at 80°C for 15 s showed mean reductions of 5.0–5.6 and 4.7–5.8 and 4.5–5.7 log₁₀ units per cm² on the skin surface and 4.4–4.6 and 3.6–4.1 and 3.2–3.4 log₁₀ units per cm² on the meat surface for *E. coli*, *S. Typhimurium* and *Y. enterocolitica*, respectively (Figure 1).

Discussion

Mean reductions of 1.0–5.8 log₁₀ units per cm² were obtained depending on the different decontamination parameters applied (Figure 1). Higher treatment temperature, longer treatment time and presence of and higher concentration of lactic acid increased microbial reductions obtained, which also have been reported in the literature (Smulders *et al.*, 1986; Dickson and Anderson, 1992; Sofos and Smith, 1998; Hugas and Tsigarida, 2008). Higher reductions (0.5–1.0 log₁₀ units per cm²) were generally observed on the skin surface compared to the meat surface and this has previously been reported (Eggenberger-

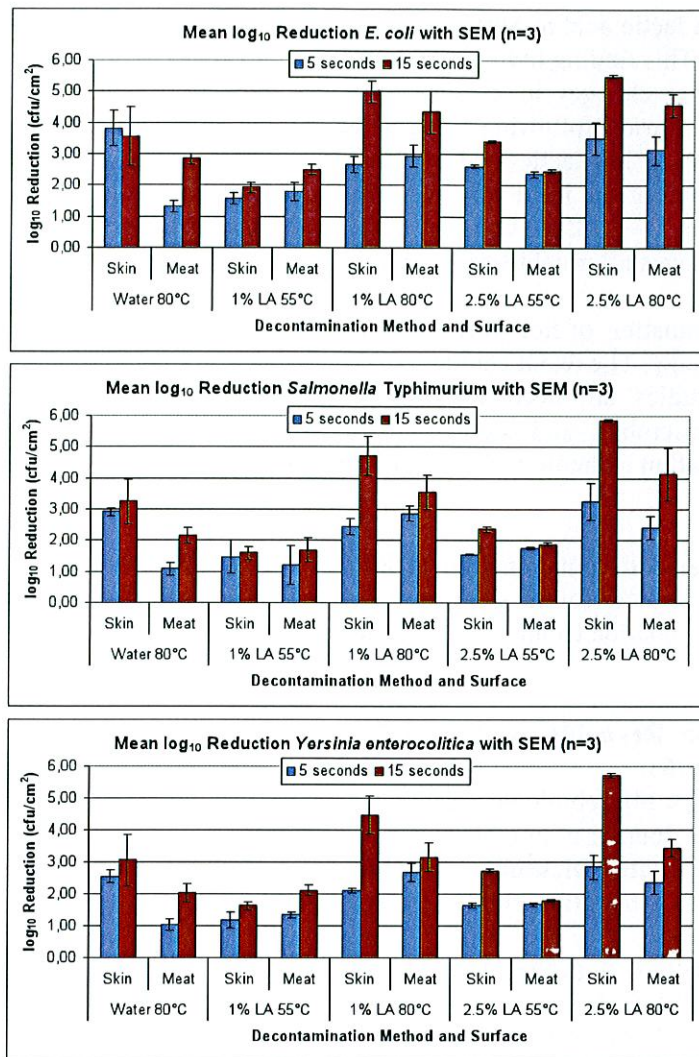


Figure 1: Effect of hot water (80°C) and 1% and 2.5% lactic acid (55°C and 80°C) decontamination for 5 and 15 s on *E. coli* (top), *Salmonella* Typhimurium (middle) and *Yersinia enterocolitica* (bottom) inoculated (10^7 cfu per cm² of each bacteria species) on skin and meat surface of pork jowls.

Solarzano *et al.*, 2002; Sommers *et al.*, 2002; Hugas and Tsigarida, 2008). The reduced effect of decontamination on the meat surface compared to the skin surface could be explained by a larger proportion of bacteria in deeper tissue structures on the meat side as demonstrated by confocal laser scanning microscopy studies (Krag, 2009, unpublished results). The observed difference between surfaces seemed to some extent equalised by application of lactic acid decontamination (Figure 1).

E. coli tended to be more sensitive to decontamination than *S. typhimurium* and *Y. enterocolitica*. In contrast, it has been reported *E. coli* 0157:H7 are more resistant to organic acids than other pathogens, e.g. *Salmonella* spp and *Yersinia enterocolitica* (Smulders and Greer, 1998).

Hot water decontamination at 80°C for 15 s showed mean reductions of 3.1-3.6 log₁₀ units per cm² on the skin surface and 2.0-2.8 log₁₀ units per cm² on the meat surface, which is in accordance with reported reductions obtained by hot water decontamination (Gill *et al.*, 1995 and 1997; Jensen and Christensen, 2000). Decontamination with 1% lactic acid at 80°C for 15 s showed mean reductions of 4.5-5.0 log₁₀ units per cm² on the skin surface and 3.2-4.4 log₁₀ units per cm² on the meat surface, showing an additive effect (1-1.5 log₁₀ units per cm²) obtained by increasing the temperature of lactic acid to 80°C. Additive effects obtained by combining different technologies for decontamination of pork meat has been studied and reported (Sommers *et al.*, 2002), including for application of sequential hot water and lactic acid decontamination (Eggenberger-Solarzano *et al.*, 2002), however the additive reductions obtained in this

study are higher. Decontamination with 1% lactic acid at 80°C for 5 s showed reductions comparable to hot water decontamination at 80°C for 15 s. This finding indicates a potential cut in water usage .

Decontamination might result in adverse changes in sensory attributes, bacterial attachment and bacterial growth after decontamination and during following refrigerated storage (Smulders and Greer, 1998; Sofos and Smith, 1998). Application of 1% lactic acid at 55° C showed minor changes in the appearance of the meat, while the appearance of the meat was negatively changed going to 2.5% lactic acid and 80° C (data not shown). Snijders et al. (1985) found that lactic acid concentrations up to 1.5% caused acceptable and reversible colour changes after cold storage, but higher acid concentrations caused irreversible effects on pork.

Decontamination of pork with the combination of hot water and lactic acid in one decontamination procedure appears to be a promising technology. The result could be microbiologically safer products with extended shelf life. However, further studies are needed in order to evaluate the effect of the decontamination treatment on sensory, microbial and storage quality of pork meat. In addition, investigation of application this decontamination technology in commercial scale are needed.

Conclusion

An additive effect of applying lactic acid decontamination at 80°C for 15 s was obtained when compared with hot water decontamination at 80°C for 15 s. If the efficiency, seen in this study, can be reproduced at the slaughter line this decontamination technology may improve food safety of pork.

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Occurrence of *Campylobacter* spp. in carcasses of pigs slaughtered for consumption in Portugal.

Morais, L. ¹, Resende, H. ¹, Fraqueza, M. J. ² & Vieira-Pinto, M. ^{1*}

¹ Dept. Ciências Veterinárias. Lab. Inspeção Sanitária. CECAV. Universidade de Trás-os-Montes e Alto Douro. Apartado 1013. 5001-911 Vila Real. Portugal. mmvpinto@utad.pt

² Dept. Produção Animal e Segurança Alimentar. Faculdade Medicina Veterinária. Polo Univ. Alto da Ajuda. Av. da Universidade Técnica. Lisboa

*Corresponding author: mmvpinto@utad.pt

Abstract

The present study was designed to determine the prevalence of *Campylobacter* spp. in pig's carcasses slaughtered for consumption in Portugal.

The present study showed that 86 % of the carcasses swab samples collected at a pig slaughterhouse were contaminated with *Campylobacter* spp. From those, 45 colonies were isolated: 96,83 % were identified as *Campylobacter coli* and 2,17% as *Campylobacter jejuni*.

This result underlines the significance of pork carcasses contamination with *Campylobacter* spp., and emphasises the importance of implementing measures in order to reduce this food borne pathogen from the pork production chain.

Further studies, using molecular tools, are being developed by the authors in order to evaluate the level of faecal contamination.

*Corresponding author: mmvpinto@utad.pt

Introduction

In 2007, Campylobacteriosis was again the most frequently reported zoonotic disease in humans in the European Union (200,507 reported confirmed cases) with the most Member States reporting an increased number of cases. Pathogenic species that cause human enteritis includes *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari*, the so-called thermotolerant campilobacters (Pearce *et al.*, 2003)

Campylobacter spp. is widely distributed in environment and is frequently found in the intestinal tracts of a wide range of domestic and wild animals (Malakauskas *et al.*, 2006). According to the EFSA report from 2007, *Campylobacter* spp. was commonly detected from live animals raised for human consumption, such as poultry, pigs and cattle (EFSA 2009).

Although it is well established that poultry meat are an important vehicle for foodborne campylobacteriosis, the involvement of pork in this foodborne disease is not well known (Pearce *et al.*, 2003). With respect to Portugal, until the present, and according to our knowledge, no information about this subject was reported or published.

Campylobacter spp. can be often found in the intestinal tract of pigs, and here *C. coli* is the more common specie identified (Nesbakken *et al.*, 2003; Malakauskas *et al.*, 2006). At the slaughterhouse, swine skin can be commonly contaminated with feces that can remain associated to the carcass as it progresses through the slaughter process. In addition, fecal material can also leak from the intestines during the evisceration process and contaminate carcass. Thus, the pathogen may be transported on contaminated carcasses from the slaughtering operation to the food-processing operation and